Use of plant extracts for the management of *Cladobotryum mycophilum* causing cobweb disease of button mushroom

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Abstract

Agaricus bisporus (Lange) Imbach, commonly known as button mushroom, is an extra nutritious and first ever globally consumed edible fungus. The perishable nature of this mushroom makes it susceptible to various fungal diseases, among which cobweb disease caused by *Cladobotryum mycophilum* (Oudem.) W. Gams & Hooz results in its quality and quantity decline. The present study was designed to use selected plant extracts: seeds of clove (*Syzygium aromaticum* L.), buds of moringa (*Moringa oleifera* Lam.) and leaves of mint (*Mentha spicata* L.) against *C. mycophilum.* Different concentrations *viz.* 1, 2 and 3% of each extract were used *in vitro* to control growth of the fungal pathogen. Results revealed that all the selected extracts inhibited fungal growth significantly. The highest concentration of clove, mint and moringa extracts inhibited the fungal growth upto 93%, 56% and 39%, respectively. Future studies focusing on *in vivo* trials are suggested for broad level application of these eco-friendly biocides against cobweb disease of button mushroom as an excellent alternative of fungicides.

Keywords: *Agaricus bisporus*, Botanicals, Cobweb, *Cladobotryum mycophilum*, *In vitro* inhibition.

Introduction

Mushrooms are one of the most abundant and inadequate sources of nutritious food consumed and cultivated globally (Gea *et al.,* 2021). They are high in fiber, vitamins, minerals, and protein, and they are also a good source of important amino acids. A cost-effective biotechnology that produces food high in protein is the cultivation of saprophytic edible mushrooms (Mehta *et al.,* 2011). Since centuries, people consume mushrooms for their flavor, medical qualities, economic importance, and biological value (Zuo *et al.,* 2016). About 12,000 different types of fungi are categorized as mushrooms, among which 2,000 are thought to be edible where *Agaricus bisporus*, commonly known as button mushroom is the most consumed and delicious edible fungi with short shelf-life (Wang *et al.,* 2015). Both the production and consumption of this mushroom have steadily increased over the last six to seven decades where China is the main exporter (Geosel *et al.,* 2014). *A. bisporus* is mostly grown commercially in Pakistan at the Margalla Mushroom Farm in Islamabad. Global mushroom production has increased more than 30-fold in the last 35 years, from 1 to 34 billion kg. Pakistani mushroom farmers made $6.90 million in total revenue by producing an estimated 155.6 kg per hectare at a gross return of Rs. 77,800 ha^{-1} (FAO, 2024).

As button mushroom is extremely delicate and perishable in nature, therefore, fungal and bacterial pathogens cause quality and quantity decline (Amin *et al.,* 2021; Carrasco *et al.,* 2017). One of the common fungal disease infecting button mushrooms is cobweb caused by *Cladobotryum mycophilum* (Lan *et al.,* 2020). Common symptoms include fine grey-white mycelium that resembles a spider web and spreads rapidly (Carrasco *et al.,* 2016). The mycelia sporulate, conidia disperse and infection spreads (Adie *et al.,* 2006). Cobweb is reported as one of the most harmful infections, reaching epidemic levels. Management of cobweb button mushroom disease with fungicides is extremely destructive practice leading severe health concerns and is banned in developed countries (Tam and Poldmaa, 2013). Moreover, a very limited number of fungicides are commercially available as a treatment of cobweb disease, including two synthetic fungicides adopted in the EU and worldwide viz: prochloraz and metrafenone (Clarke *et al.,* 2024). The application of chemical management practices causes toxic effects, therefore, application of bio-practices focusing plant-based material is recommended globally for the control of fungal pathogens (Khan and Javaid, 2020; Khan *et al.,* 2021; Jabeen *et al.,* 2022a). To optimize disease control, chemical use should be integrated with other cultural practices and hygiene enhancement measures (Back *et al.,* 2012; Kim *et al.,* 2014). Bio-management using biocontrol agents, plant extracts, essential oils sprays, are all excellent eco-friendly practices adopted against fungal diseases of mushrooms worldwide (Clarke *et al.,*

2022). The present study was designed focusing clove, moringa and mint extracts application against this deteriorating disease of button mushrom, with the objective to enhance shelf-life and produce disease free healthy mushroom for consumption.

Materials and Methods

Collection of diseased samples

Samples of diseased specimens were collected from three selected locations *viz.* Mushroom Cultivation Laboratory PMAS Arid Agriculture University Rawalpindi, Carwan Mushroom Farm Quetta, and Margalla Mushroom Farm Islamabad, Pakistan. Samples were collected from the 3rd flush, approximately after 20 days of fruiting (Fig. 1). The polythene bags were used for sampling to retain moisture for further analysis. Disease incidence (DI) measurement at selected sites was determined using following formula:

$$\text{DI (\%)} = \frac{\text{No. of infected bags}}{\text{Total no. of bags}} \times 100$$

Isolation of fungal pathogen

The pathogen was cultured on potato dextrose agar (PDA) and the infected mushroom portions were surface sterilized in ethanol, washed in distilled water and the cottony mycelium was placed on the growth media in sterile Petri plates, followed by spreading the casing layer directly on the PDA and incubated at 25 ºC for 7 days. Morphological studies were carried out focusing colony color, texture, spore shape and size (Seifert and Gams, 2011). The isolated fungus was further subjected to management experiment using selected plant material *viz.* leaves and stem of moringa, leaves of mint and buds-seeds of clove, collected from local herbal store of Rawalpindi, Pakistan. The selected plant materials were dried and crushed to prepare plant extracts. The leaves of moringa and mint, and seeds of clove were rinsed with sterile water and then shade dried to remove excessive moisture content. The dried botanicals were grounded by using electric grinder into a fine powder and 30 g of each botanical was soaked into 300 mL of solvents (methanol, ethanol and aqueous) in conical flask and hand shaken to mix thoroughly. The suspension was filtered through Whatman filter paper number 1 and the extract obtained was kept under shade to allow evaporation of excessive ethanol. After 3–4 days, the ethanol was evaporated completely leaving behind the sticky residue. The extract was weighed on an electric weighing balance and 7 g extract of each botanical was added to plastic centrifuge tubes. A total of 17.5 mL distilled water was added to dilute the final stock solution at 40% concentration, respectively (Jabeen *et al.*, 2022b).

Antifungal bioassays

To evaluate the effect of botanical extracts against the *Cladobotryum mycophilum*, poisoned food technique was used, and the prepared botanical extracts were added to the three different falcon tubes containing 100 mL autoclaved PDA to make three concentrations 1, 2 and 3% by adding the 3, 6 and 9 mL of the stock solution, respectively. The poisoned media was mixed thoroughly, poured into sterile media plates, and allowed to solidify. The entire experiment was carried out in a laminar flow chamber to avoid contamination. The uniformly grown mycelial discs of *C. mycophilum* were taken with the help of a cork borer (3 mm) and placed at the center of media plates of different concentrations. All treatments of botanicals and fungicide (fludioxonil) were applied each with three replicates along with control and the plates were carefully sealed with the parafilm to avoid contamination and were placed in the incubator at 22–25 ºC. The growth of each mycelium of *C. mycophilum* was measured with the help of a scale from the mycelial disc and the readings were taken after 3, 5 and 7-days intervals of the inoculation from equal sides and average was calculated. To observe the percent inhibition of *C. mycophilum*, the following formula was applied:

$$\text{Inhibition (\%)} = \frac{C-T}{C} \times 100$$

Where C is the growth in the control plate and T is the mycelial growth inpoisoned plates.

Statistical analysis

The results obtained were statistically analyzed by SPSS 20 following completely randomized design two-way ANOVA and LSD at 5% level of significance.

Results and Discussion

The pathogen was identified as *C. mycophilum* based on morphological study. *C. mycophilum* was recorded from two locations namely Carwan mushroom farm Quetta and Margalla mushroom farm Islamabad. Cobweb symptoms were observed at third flesh after 15–21 days of fruiting of button mushroom. On 10th day of fruiting, there was no visible disease incidence recorded at all selected sites. Whereas on 20th day of button mushroom fruiting, 80% disease incidence was reported at Quetta Carwan mushroom farm, followed by 30% disease incidence observed at Margalla mushroom farm Islamabad, and no incidence of cobweb disease was recorded at mushroom cultivation laboratory PMAS-AAUR, respectively.

Effect of botanicals on growth of *C. mycophilum* on 3rd day

The results revealed that among all applied plant extracts as management practices, clove extracts were recorded highly affective where the fungal radial growth decreased with higher concentrations: 0.97 cm at 1%, 0.58 cm at 2%, and 0.18 cm at 3%. Whereas application of moringa extract was recorded with a reduced radial growth at all concentrations viz: 2.68 cm at 1%, 2.40 cm at 2%, and 2.27 cm at 3%, respectively. The mint extracts application was recorded somehow similar to moringa, where the radial growth decreased with concentration: 2.10 cm at 1%, 1.78 cm at 2%, and 1.62 cm at 3%. Fungicide consistently showed the lowest radial growth of 0.49 cm across all concentrations, indicating significant effectiveness at $P \leq 0.05$ (Fig. 1).

The fungicide was the most effective treatment with residual effect, consistently inhibiting pathogen growth at all concentrations. It is pertinent to mention that fungicides hold significant antifungal properties compared to botanical extracts, but on the other hand they are extremely toxic to health and are strongly prohibited at harvesting stage. Clove as a bio-control agent showed a significant reduction in radial growth with increasing concentrations, indicating it is effective, with zero hazardous effect to human and animal health. Both moringa and mint extract reduced pathogen growth compared to the control but were less effective compared to clove. Moringa had slightly better performance at lower concentrations compared to mint extracts. The control had the highest radial growth, confirming that the treatments were effective in reducing pathogen growth (Table 1).

Effect of botanicals on growth of *C. mycophilum* on 5th day

Our findings focus the significant differences ($P \leq 0.05$) in the effectiveness of the treatments. The fungicide consistently exhibited the lowest radial growth (0.67 cm) across all concentrations, compared to the control group, which showed a radial growth of 4.45 cm. Clove extract inhibited cobweb growth efficaciously, with radial growth reduction range 1.33 cm at 1% to 0.30 cm at 3%. Moringa and mint extracts application was observed with moderate effectiveness *viz.* 3.42 cm at 1%, 3.01 cm at 2% followed by 2.51 cm at 3%, whereas mint reduced the radial growth up to 2.63 cm at 1%, 2.35 cm at 2%, and 1.87 cm at 3%, respectively. The fungicide's superior performance can be attributed to its strong and consistent inhibitory action, but it is pertinent to mention that fungicides hold extremely hazardous effects causing toxins to human health and environment. Clove extract, stands out as the best biocontrol option, offering an eco-friendly natural alternative with substantial pathogen suppression. Moringa and mint extracts, though less effective individually, could complement other control measures within an integrated approach (Table 2).

Effects of botanicals on growth of *C. mycophilum* on 7th day

All the treatments had a significant impact ($P \leq 0.05$) on the radial growth of pathogen. The fungicide showed the lowest radial growth at 0.78 cm across all concentrations, compared to the control group, which had a radial growth of 4.50 cm. Clove extract demonstrated significant efficacy, with radial growth values decreasing from 2.73 cm at 1% to 0.48 cm at 3%. Mint showed moderate effectiveness, with radial growth ranging from 2.90 cm at 1% to 1.98 cm at 3%. Moringa exhibited the least reduction in radial growth, with values of 4.48 cm at 1%, 3.60 cm at 2%, and 2.77 cm at 3% (Table 3).

Excessive use of chemicals against fungal diseases of mushrooms results extreme pollution and toxicity with harvest detrimental to human health. The notable alternative is to apply eco-friendly plant extracts with zero residual effect (Majumder *et al.,* 2024). Clove, mint and moringa hold great antifungal attributes, enhancing shelf-life of perishable mushrooms. Our findings revealed that clove extract was most efficacious in inhibiting the mycelial growth of *C. mycophilum* at different days interval, followed by moringa and mint extracts. El-Mohamedy and Abdallah (2014) reported similar results were moringa leaf extract inhibited mycelial growth of various opportunistic plant fungal pathogens. There were differences in antifungal activity of various plant extracts using ethanol and methanol extraction, as both these solvents extract active ingredient from plants more precisely (Nalubega *et al.,* 20211). Another, study revealed efficacy of plant extracts against button mushrooms fungal diseases, where *in vivo* application of neem completely inhibited the mycelium of *Verticillium fungicola* after 7th day post inoculation (Singh *et al.,* 2016). Our study contradicts here, as neem extract is bitter in taste and its application at mushroom maturity stage, results in taste alteration, hence resulting compromised flavor. Our study emphasizes clove extract as the most effective bio-management agent against cobweb disease. The study conducted by Pinto *et al.* (2009) support our research, where clove causes considerable reduction in the quantity of ergosterol, a specific fungal cell membrane component, and enhanced shelf life of perishable agricultural produce. Apart from inhibiting fungal mycelial growth, clove, moringa and mint extracts were proven effective against fungal disease dispersal.

Conclusion

The present research summarizes the impact of plant extracts against world's most devastating fungal pathogen of button mushroom. Clove extracts

were experimentally proven most efficacious antifungal agent against cobweb disease of button mushroom. The presence of natural volatile compounds in cloves, disrupts fungal cell-wall and hence act as an excellent bio-management. Moreover, these biocides are conducive to environment with zero hazardous effects toward humans and animals. The findings of this research revealed that all applied extracts have antifungal activity against the examined pathogen to varying degrees, with no hazardous effects. It is recommended that the cost-effective alternative biocidal treatment against these deteriorating fungal rots must be seriously considered as an alternative to toxic fungicides and practically be implemented for production of diseased free heathy nutritious mushrooms for human consumption.

Novelty statement

Our study highlights the novel application of clove extract as a biofungicide, showcasing its efficacy in controlling cobweb disease and enhancing button mushroom yield without compromising quality.

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Contribution of authors

RU performed experiments and wrote original draft, GI analyzed data, GLK performed editing and proofreading, MIH and AM critically reviewed the manuscript.

Conflict of interests

No potential conflict of interest is declared by the authors.

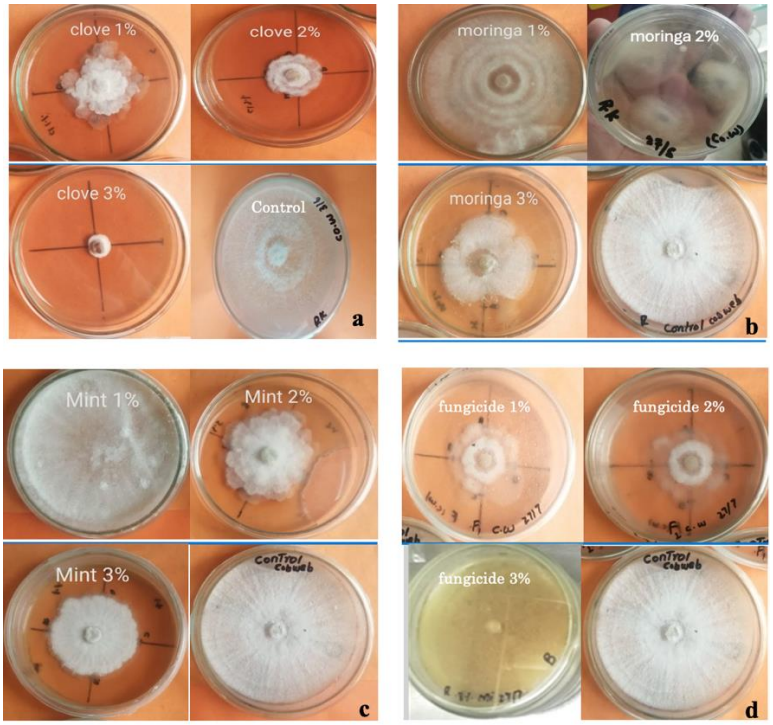

Fig. 1: Fungal growth inhibition by different concentrations of (a) clove, (b) moringa, (c) mint and (d) fungicide application.

Table 1: Effect of different concentrations of botanical extracts on radial growth of *Cladobotryum mycophilum* on 3rd day after incubation.

Treatment	Radial growth of *C. mycophilum* (cm)			
	1%	2%	3%	control
Clove	0.97 f	0.58 g	0.18 h	3.28 a
Moringa	2.68 b	2.40 c	2.27 cd	3.50 a
Mint	2.10 d	1.78 e	1.62 e	2.91 a
Fludioxonil	2.21 g	2.35 g	2.41 g	3.27 g

Values with different letters show significant difference at $P \leq 0.05$.

Table 2: Effect of different concentrations of botanical extracts on radial growth of *Cladobotryum mycophilum* on 5th day after incubation.

Treatment	Radial growth of *C. mycophilum* (cm)			
	1%	2%	3%	control
Clove	1.33 g	0.92 h	0.30 j	3.66 a
Moringa	3.42 b	3.01 c	2.51 de	4.43 a
Mint	2.63 d	2.35 e	1.87 f	3.99 a
Fludioxonil	1.08 i	1.11 j	2.82 j	3.89 i

Values with different letters show significant difference at $P \leq 0.05$.

Table 3: Effect of different concentrations of botanical extracts on radial growth of *Cladobotryum mycophilum* on 7th day after incubation.

Treatment	Radial growth of *C. mycophilum* (cm)			
	1%	2%	3%	control
Clove	2.73 cd	1.80 e	0.48 g	4.50 a
Moringa	4.48 a	3.60 b	2.77 cd	3.99 a
Mint	2.90 c	2.63 d	1.98 e	3.51 a
Fludioxonil	0.79 h	1.52 f	2.02 c	3.61 c

Values with different letters show significant difference at $P \leq 0.05$.

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